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13. ABSTRACT ***(Maximum 200 Words)***

The changes in the gene program of neoplastic cells that regulate the expression of an invasive phenotype are largely undefined. Direct comparisons of the gene expression profile displayed in normal and carcinomatous breast tissues have provided insights into the mechanisms underlying tumor progression. However, attempts to identify the gene products differentially expressed during invasion *in vivo* have been hampered by the fact that only a small percentage of the cells recovered from a tumor mass are actively engaged in invasive behavior at the time of isolation. Because tissue remodeling induced during mammary gland involution bears homology to early stages of carcinogenesis, the involuting mammary gland may be used to identify genes that control matrix turnover in cancerous states. To this end, we propose to *i) screen involuting versus resting mouse mammary glands by oligonucleotide microarray for differentially expressed gene products associated with matrix remodeling, ii) evaluate potential role of differentially expressed gene products in regulating cell invasion in vitro and/or in vivo and iii) evaluate role of differentially expressed gene products in regulating breast cancer cell invasion.*

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Table of Contents

I. INTRODUCTION

Current evidence suggests that breast carcinoma cells invade local tissues and metastasize by i) altering their cell-cell and cell-matrix interactions, ii) displaying an aberrant motile phenotype, and iii) either synthesizing, or inducing the synthesis of, proteolytic enzymes that degrade the structural barriers established by the extracellular matrix[1-3]. The complex changes in the gene program of neoplastic cells that regulate the expression of this phenotype are largely undefined, but increased interest has focused on identifying those genes that are specifically overexpressed in human breast cancer[(e.g., 3-10)]. Such information not only provides new insights into the cellular factors that control tissue-invasive behavior, but may also lead to improvements in patient diagnosis and to the more rational design of therapeutic interventions[3-10]. Consistent with this rationale, direct comparisons of the gene expression profile displayed in normal versus neoplastic breast cancer cell lines, or normal and carcinomatous breast tissues, have provided a number of novel insights into the mechanisms and processes underlying tumor progression[6-11]. Interestingly, despite the power of the analytical techniques employed for these purposes, the number of differentially expressed genes identified thus far are - at first glance - perplexingly small, despite the striking changes known to occur in cellular behavior[(e.g., 7,8)]. However, analyses of breast cancer cell lines grown *in vitro* or static tumor masses recovered from *in vivo* sites of disease may be problematic. First, comparisons between normal and neoplastic breast cancer cell lines grown atop plastic substrata *in vitro* will not recapitulate the complex interactions known to occur across the carcinoma-mesenchymal cell axis *in vivo*[1,2]. Indeed, many of the most interesting gene products that have been associated with the expression of tissue-invasive phenotypes in breast cancer tissue are synthesized by surrounding stromal cells rather than the tumor itself[2,3,10]. Secondly, while the gene expression patterns identified in tissues recovered from *in vivo* sites clearly circumvent the limitations inherent in the *in vitro* studies, only a small percentage of the cells recovered from a tumor mass at a single, fixed time point would be expected to be actively engaged in invasive behavior. Given the many similarities between developmental/tissue repair processes and malignant growth (re; the ability of cancer cells inappropriately recapitulate developmental programs associated with epithelial-mesenchymal cell transitions or repair programs associated with wound healing[12,13]), we have considered the possibility that the *in situ* induction of a synchronous matrix remodeling program in normal tissues would allow for the more efficient isolation of those gene products critical to cancer cell invasion. Indeed, recent studies have demonstrated that gene expression patterns associated with the tissue remodeling program induced during the involution of the normal lactating mammary gland bear considerable overlap with those detected in the early stages of carcinogenesis (e.g., stromeylsin-1, stromeylsin-3, urokinase-type plasminogen activator, tissue inhibitor of metalloproteinases[14-16]). Hence, we propose to use the involuting mammary gland explant model as a means to rapidly enrich for, and identify, the subset of genes that control the disassembly of the extracellular matrix in cancerous states. Furthermore, by selectively identifying the subset of gene products that regulate invasion of breast cancer cells, new diagnostics as well as novel targets for therapeutic intervention can be rapidly identified.

II. BODY

In the original proposal, we intended to generate cDNA libraries from control versus involuting mammary gland explants. However, the high rate of cell apoptosis in control glands precluded

analysis and emphasis was shifted to analyses of gene expression patterns in tissues recovered from lactating versus involuting glands *in vivo* by oligonucleotide array (see approved "Revised Statement of Work"). Each of the proposed aims have now been completed as described below.

Task 1. Screen involuting versus resting (i.e., lactating) mammary glands by oligonucleotide array for differentially expressed gene products associated with matrix remodeling.

a. Documentation of involution program in mammary explants. To demonstrate that an involution program was successfully engaged in mammary gland tissue, glands were excised from lactating (day 10) or involuting glands (day 3 post-weaning). Tissue sections were then processed for H and E staining, apoptosis (as assessed by TUNEL staining) or for the dissolution of the subepithelial basement membrane by immunofluorescence (as assessed by staining for type IV collagen or laminin). As shown in Figure 1, the milk-engorged ductal system of lactating mice rapidly collapsed 3 days post-weaning. Coincident with this event, an increase in apoptosis was noted which paralleled a major loss and/or fragmenting of basement membrane-associated type IV collagen or laminin (Fig. 1). As the matrix-degrading involution program had been successfully engaged, tissues from the mice were collected and subjected to oligonucleotide array analysis as described below.

b. Collection mRNA and performance of oligonucleotide microarray analysis. In three experiments, RNA was isolated from glands with a Qiagen RNassay mini-kit and cRNA prepared for hybridization as described[17,18]. Oligonucleotide arrays (Gene Chip, Affymetrix) representing a total of 30,000 EST cluster sequences and/or full-length genes were used for hybridization according to the manufacturer's instructions. Arrays were then scanned using an Affymetrix confocal scanner and analyzed using Gene Chip 3.0 Software (Affymetrix). Expression data from the Affymetrix arrays were analyzed using a statistically based analysis methodology that estimates expression levels and provides confidence intervals for these estimates. It also allows for the normalization of array-based expression data to control for variations due to non-biological factors such as array-to-array variability, and variations in sample quality. For each gene, the presence or absence of a transcript was determined by testing the Null hypothesis. Briefly, the arrays included a set of probes derived from non-eukaryotic ("foreign") organisms (e.g., bacterial and bacteriophage sequences) which were defined as the "null set". This null set thus defines the intensity of nonspecific background/cross-hybridization. This null intensity distribution is modeled by a parametric statistical distribution. Since intensity is a positive random variable, this null distribution is modeled by either a Gamma or a Weibull class distribution. Once the parametric null distribution is determined, we computed the p-value for the hypothesis that the observed hybridization intensity values are also a random sample from the null distribution. Target genes with low p-values (i.e., not likely to have come from the same distribution as the null genes) are classified as present. The p-value provides a continuous measure of the confidence in the presence of a gene in the target sample. We also include a mathematical method to standardize the gene-expression levels between different samples, based on exogenous gene spikes, added at known concentrations, that constitute a calibration set[18]. Genes scored as "positive" (i.e., induced) in involuting tissues were i) more highly expressed in each of 3 independently performed experiments and ii) expressed at levels ≥ 2.5 than those detected in lactating glands in at least 2 of the 3 experiments.

Following analysis of the approximately 100 genes whose expression was upregulated as defined, a series of proteolytic enzymes were identified (Appendix I). In the matrix metalloproteinase family, stromelysin-1 was the only upregulated member whose expression reached significance (Appendix I). However, the membrane-anchored metalloproteinase, MT1-MMP, was also identified though its expression level did not increase by the requisite 2.5-fold in 2/3 samples. However, given the positive trend, samples from involuting tissues were examined by RT-PCR at both 3 and 5 days post-weaning. Significantly, MT1-MMP mRNA was dramatically upregulated at the latter time point (Fig. 2). In addition, message levels for three matrix-destructive cathepsins were also found to be upregulated during involution, i.e., the aspartyl proteinase cathepsin D, and the cysteinyl proteinases, cathepsins L and S.

Table I
Fold-Increase in Gene Expression

	EXP #1	EXP #2	EXP #3
cathepsin D	4.7	3.9	2.3
cathepsin L	4.1	2.9	1.8
cathepsin S	7.4	6.2	2.3

<u>Task 2.</u> Evaluate the potential role of differentially expressed gene products in regulating cell invasion in vitro and/or in vivo.

With regard to the MMPs, both stromelysin-1 and MT1-MMP are expressed in breast cancer[19,20]. Thus, we sought to determine whether either of these enzymes might serve as basement membrane-degrading enzymes. To this end, we developed a model system wherein an immortalized epithelial cell line deposits an intact basement membrane *in vitro* (containing type IV collagen, laminin and heparan sulfate proteoglycan; data not shown) atop a dense layer of type I collagen (Fig. 3). Following lysis of the overlying epithelium, COS-1 cells that have been engineered to overexpress human stromelysin-1 were then allowed to adhere to the denuded basement membrane. Following a 5 d incubation period, the basement membrane retained its normal structure as assessed by transmission electron microscopy (Fig. 4). However, in marked contrast, cells transfected with MT1-MMP not only rapidly degraded the underlying substratum, but also conferred the recipient cells with invasive activity (Fig. 5). While MT1-MMP could conceivably mediate basement membrane degradation directly, the proteinase can also process the MMP zymogen, gelatinase A (or MMP-2), to its active form[21]. Further, gelatinase A has been posited to serve as a basement membrane degrading enzyme itself[22]. However, COS-1 cells do not express gelatinase A and the experiments described above were performed in the absence of exogenous gelatinase A (i.e., serum contains gelatinase A, but this can be removed by gelatin-affinity chromatography)[21]. Nonetheless, to rule out a role for gelatinase A directly, COS-1 cells were stably transfected with gelatinase A and cultured atop the denuded basement membrane as described. While no change in basement membrane structure was noted (data not shown), the cells only secreted gelatinase A in its latent form (see below). To test the ability of active gelatinase A to exert proteolytic activity in this system, a chimeric enzyme was designed wherein a basic recognition motif (RXKR, where R = Arg, X = any amino acid, K = Lys) for the proprotein convertase, furin, was inserted directly upstream of the catalytic domain of gelatinase A between N^{109} and Y^{110} (we described this general approach previously for other MMPs in ref.

23). In this manner, progelatinase is cleaved within the *trans*-Golgi network to a fully active form prior to secretion. Indeed, as shown in Figure 6, supernatants of transfected cells released active gelatinase A as assessed by gelatin zymography. Nonetheless, cells overexpressing active gelatinase A did not display basement membrane-degrading activity (Fig. 6). Likewise, the closely related gelatinase, MMP-9, was also unable to degrade the underlying basement membrane when expressed in its fully active form (the basic recognition motif was inserted between R^{106} and F^{107}; Fig. 6). Thus, only MT1-MMP, an MMP upregulated during the involution cycle, arms expressing cells with the ability to degrade the basement membrane construct used in these studies. Nonetheless, as the basement membrane was generated *in vitro*, concerns could be raised that basement membranes assembled *in vivo* display unique properties relative to their susceptibility to degradation. Consequently, we have extended these studies to analyze the ability of MT1-MMP to degrade basement membrane recovered from animal tissues. As shown in Figure 6, an intact basement membrane is located beneath the mesothelial cell layer of the peritoneum, Hence, resident cells were lysed by freeze-thawing and the denuded peritoneal basement membrane overlaid with MT1-MMP expressing COS-1 cells. As predicted, the MT1-MMP transfected cells displayed a proteolytic activity similar, if not identical, to that observed with the "*in vitro*" basement membrane constructs (Fig. 7). Further, the invasive/degradative activity was completely blocked in the presence of the synthetic MMP inhibitor, BB-94 (Fig. 7).

While cathepsin D has been previously associated with human breast cancer and shown to express matrix destructive activity *in vitro*[24,25], roles for cathepsins L and S in regulating extracellular matrix turnover are less clear. Though these enzymes are usually confined to the lysosomal system (they are unstable at neutral pH), we have recently characterized cathepsin L and S as matrix-destructive enzymes in human macrophages[26]. However, whereas macrophages are able to secrete the active enzymes extracellularly into an acidic pericellular environment maintained by H^+-pumps targeted to the plasma membrane, we have been unable to identify other cell types capable of displaying this phenotype[26]. As such, we have been unable to demonstrate the ability of cathepsin L or S to confer degradative activity in transfected COS cells. Given this limitation, we instead attempted to determine whether the involution program in cathepsin L/S-deleted mice was affected. However, these animals did not breed well in our facility which precluded attempts to study the effects of the L/S-deficient state on the involution program. Further, in the absence of direct evidence to support their extracellular secretion from the mammary epithelium (or tumor cells), we would not be able to rule out the possibility that the proteinases were only required for intracellular, lysosomal degradation and not extracellular proteolysis.

<u>Task 3.</u> Evaluate the role of differentially expressed gene products identified in the involution screen to regulate breast cancer invasion.

To determine the ability of MT1-MMP to regulate tumor cell invasion, we first examined the ability of invasion-incompetent MCF-7 cells to penetrate the basement membrane before or after transfection with MT1-MMP. As shown in Figure 8, MCF-7 breast cancer cells seeded atop the basement membrane construct did not display invasive activity as assessed by phase contrast microscopy or in H-E sections. However, following transfection with MT1-MMP (but not stromelysin-1), the cells displayed striking invasive activity (Fig. 8). As human breast cancer tissue expresses MT1-MMP *in vivo*[27], the invasive activity of MT1-MMP-positive tumor cells

were examined (i.e., the MB-231 cell line). Significantly, when those cells were cultured atop the peritoneal basement membrane, the underlying matrix was degraded (as assessed by scanning electron microscopy; Fig. 9). In the presence of recombinant TIMP-2, a potent MT1-MMP inhibitor, degradation and invasion were completely blocked over a 7 d assay period (Fig. 9). In preliminary experiments performed with a neutralizing monoclonal antibody directed against MT1-MMP, invasive activity was likewise inhibited completely. Taken together, these data suggest that MT1-MMP may be the critical proteinase involved in basement membrane degradation and invasion by breast carcinoma cells.

III. KEY RESEARCH ACCOMPLISHMENTS

- Gene program associated with mammary gland involution program characterized.
- Membrane type-1 matrix metalloproteinase identified as key basement membrane degrading enzyme.
- Membrane type-1 matrix metalloproteinase identified as pro-invasive factor for human breast cancer cells and potential target for therapeutic intervention.

IV. REPORTABLE OUTCOMES

- Presentation at Gordon Conference on Matrix Metalloproteinases, 2001.
- New funding obtained to assess role of membrane-type matrix metalloproteinases in breast cancer development from Komen Breast Cancer Foundation.
- Manuscript in preparation, "Regulation of Basement Membrane Invasion by MT1-MMP", Kevin Hotary and Stephen J. Weiss.

VI. CONCLUSIONS

With the identification of suitable mammary gland tissues for isolating gene products differentially expressed during matrix-remodeling events, the model system has been used to identify genes that likely control the disassembly of the matrix during tumor invasion and metastasis. Furthermore, by selectively identifying critical gene products that regulate invasion, new diagnostics as well as novel targets for therapeutic intervention may be identified.

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VIII. APPENDICES

Bibliography - None

Personnel
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Kevin Hotary, Ph.D.

Figure Legends

Figure 1. Induction of Mammary Gland Involution Program. Lactating (10 days) or involuting (3 days post-weaning) glands were isolated from wild-type mice and tissues processed for H and E staining and apoptosis (TUNEL) as well as immunostaining for laminin and type IV collagen as described[27]. Lactating glands were milk-engorged and showed no significant apoptosis. In addition, basement membranes were laminin- and type IV collagen- positive. Following 3 days of involution, glandular structures collapsed and apoptotic cells were observed as yellow-green staining. Laminin staining decreased significantly while type IV collagen immunoreactivity decreased coincident with a thickening/blurring of basement membrane structure.

Figure 2. RT-PCR Analysis of MT1-MMP Expression in Mouse Mammary Glands. RNA was isolated from virgin, 10 d lactating, 1 d involuting or 5 d involuting glands. Reverse transcription was performed as described[28]. The arrow at left marks the position of the MT1-MMP standard.

Figure 3. Basement Membranes Synthesized by MDCK Epithelial Cells are Deposited Atop a 3-Dimensional Type I Collagen Gel During a 3 Week Culture Period. In panel A, TEM analysis shows an epithelial cell depositing a ~90 nm thick basement membrane which is more readily observed after the overlying cell layer has been lysed (panel B). Panels C and D are scanning electron micrographs showing the type I collagen gel upon which the intact basement membrane is deposited, respectively.

Figure 4. Basement Membrane-Invasive Potential of Stromelysin-1. Stromelysin-1 transfected cells cultured atop an intact basement membrane (arrows) were unable to confer degradative or invasive activity as visualized by transmission electron microscopy.

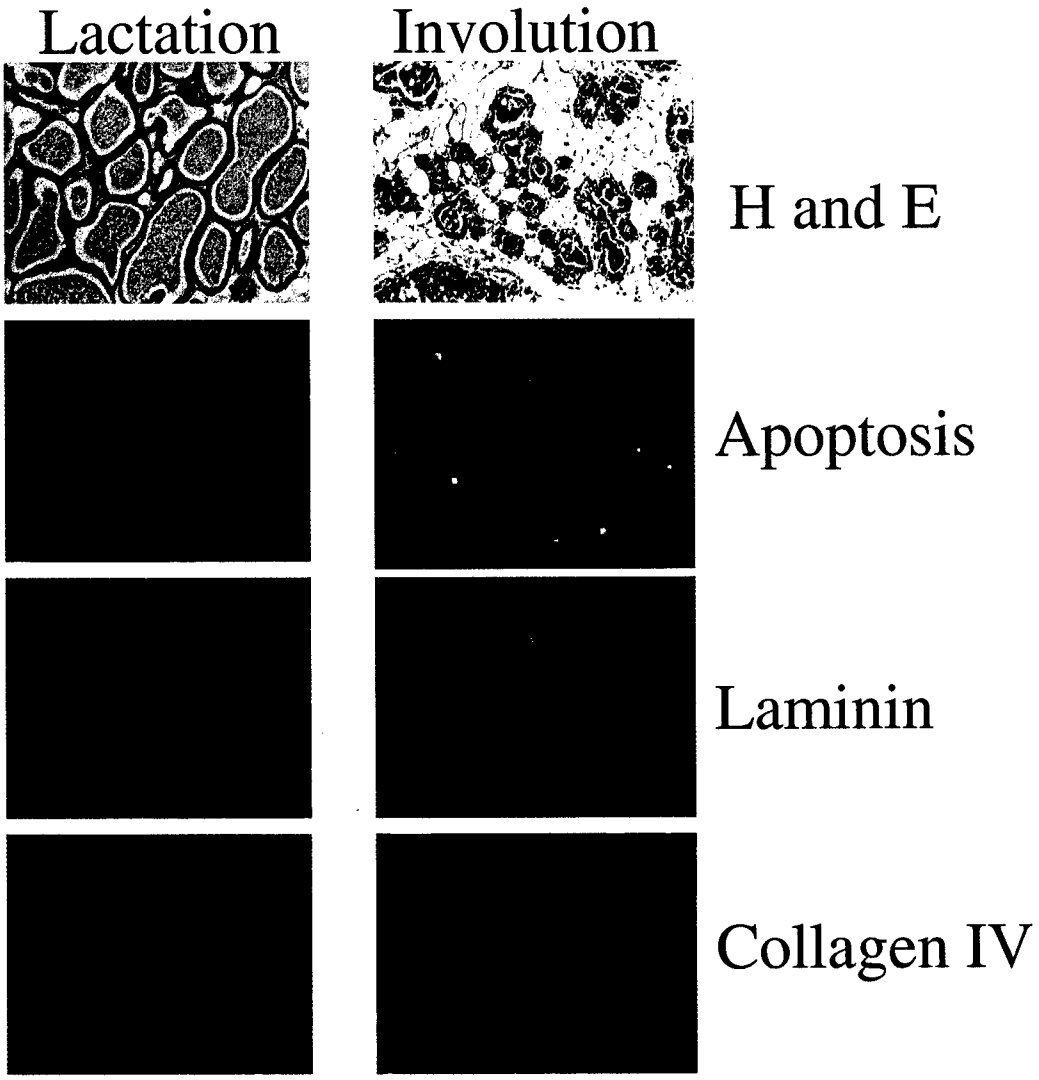
Figure 5. MT1-MMP-Dependent Basement Membrane Degradation/Invasion. Control cells did not penetrate the basement membrane as assessed by TEM or SEM analysis following a 5 d incubation period. (upper left and right panels). In contrast, MT1-MMP-transfected cells perforated the BM in representative TEM and SEM samples, respectively. Arrows indicate the position of the basement membrane.

Figure 6. Active MMP-2 or MMP-9 Do Not Confer Basement Membrane-Invasive Activity. COS-1 cells were transfected with MMP-2 or MMP-9 chimeric constructs and supernatants analyzed for MMP activation by gelatin zymography (yellow arrow in left panel depicts position of proenzyme and the red arrow marks the position of the fully active, processed enzyme) or for basement membrane degradation (right side) by scanning electron microscopy.

Figure 7. MT1-MMP-Dependent Degradation of an In Vivo-Generated Basement Membrane. In the upper panel, the ~90 nm thick peritoneal basement membrane is visualized by TEM. In the three lower panels, SEM analysis demonstrates that MT1-MMP-transfected cells, but not control vector transfectants, degraded the underlying basement membrane by a process that was blocked by the synthetic MMP inhibitor, BB-94 (5 μM).

Figure 8. MT1-MMP Regulates Basement Membrane Invasion of Transfected MCF-7 Cells. Wild-type or MT1-MMP-transfected MCF-7 cells were cultured atop a basement membrane construct which overlaid a 3-D type I collagen gel in a Transwell insert as described. Following a 5 d incubation period, invasion by MT1-MMP transfectants was observed by phase contrast microscopy (left panel) or in H and E sections (right panel). The control MCF-7 cells which do not express MT1-MMP, did not display invasive activity.

Figure 9. Basement Membrane Invasion by Human MB-231 Breast Cancer Cells. Peritoneal basement membranes (top panel) were used as a substratum for MB-231 cells during a 5 d incubation period. Cells were then lysed and the underlying basement membrane viewed by scanning electron microscopy. MB-231 cells left large holes in the basement membrane leaving the underlying interstitial matrix exposed (middle panel). Culturing the MB-231 cells atop the basement membrane in the presence of the MMP inhibitor, TIMP-2 (5 μg/ml), left the basement membrane largely intact (bottom panel).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Involution
H and E
Apoptosis
Laminin
Collagen IV

Figure 1

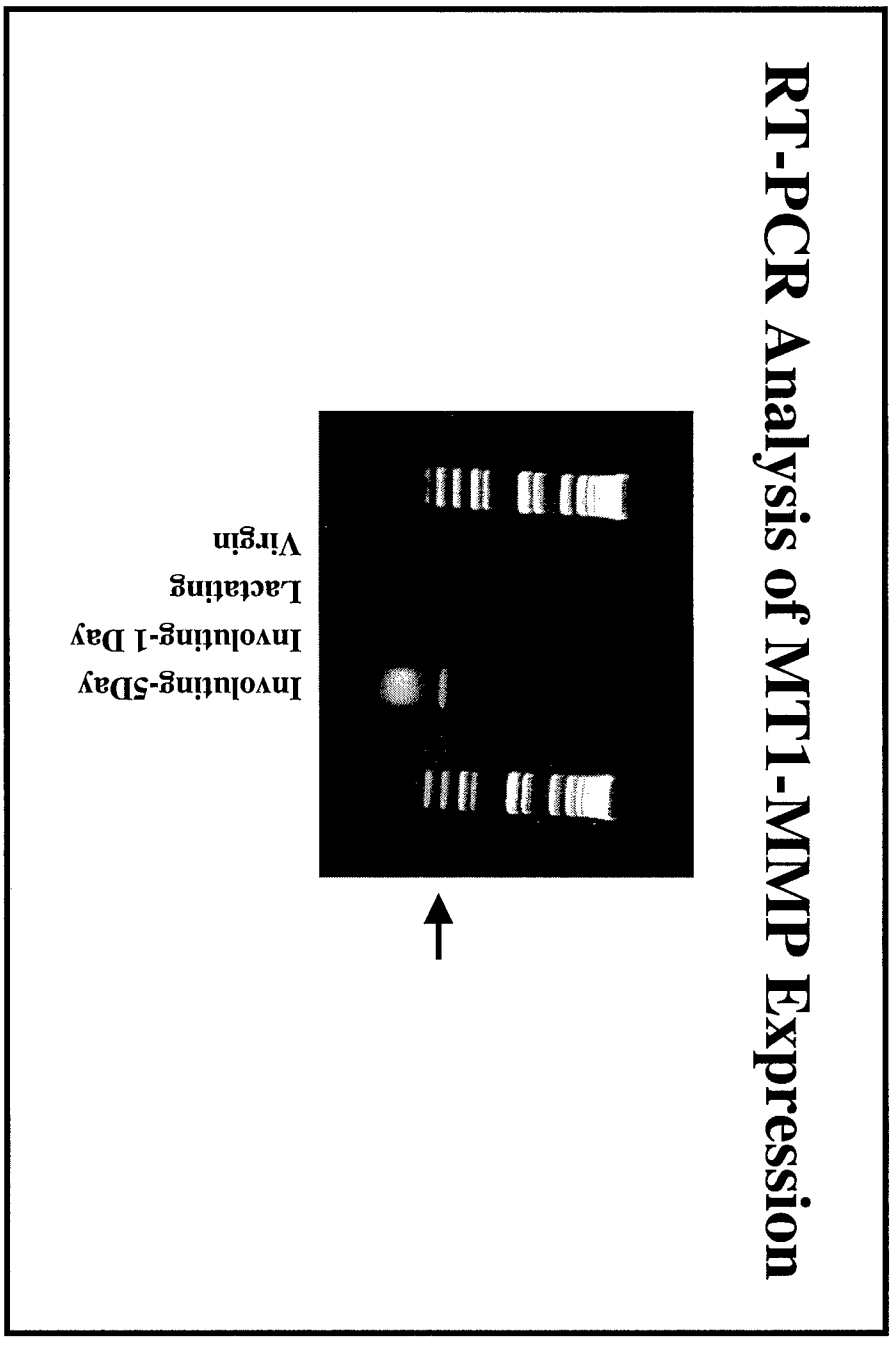
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Figure 2

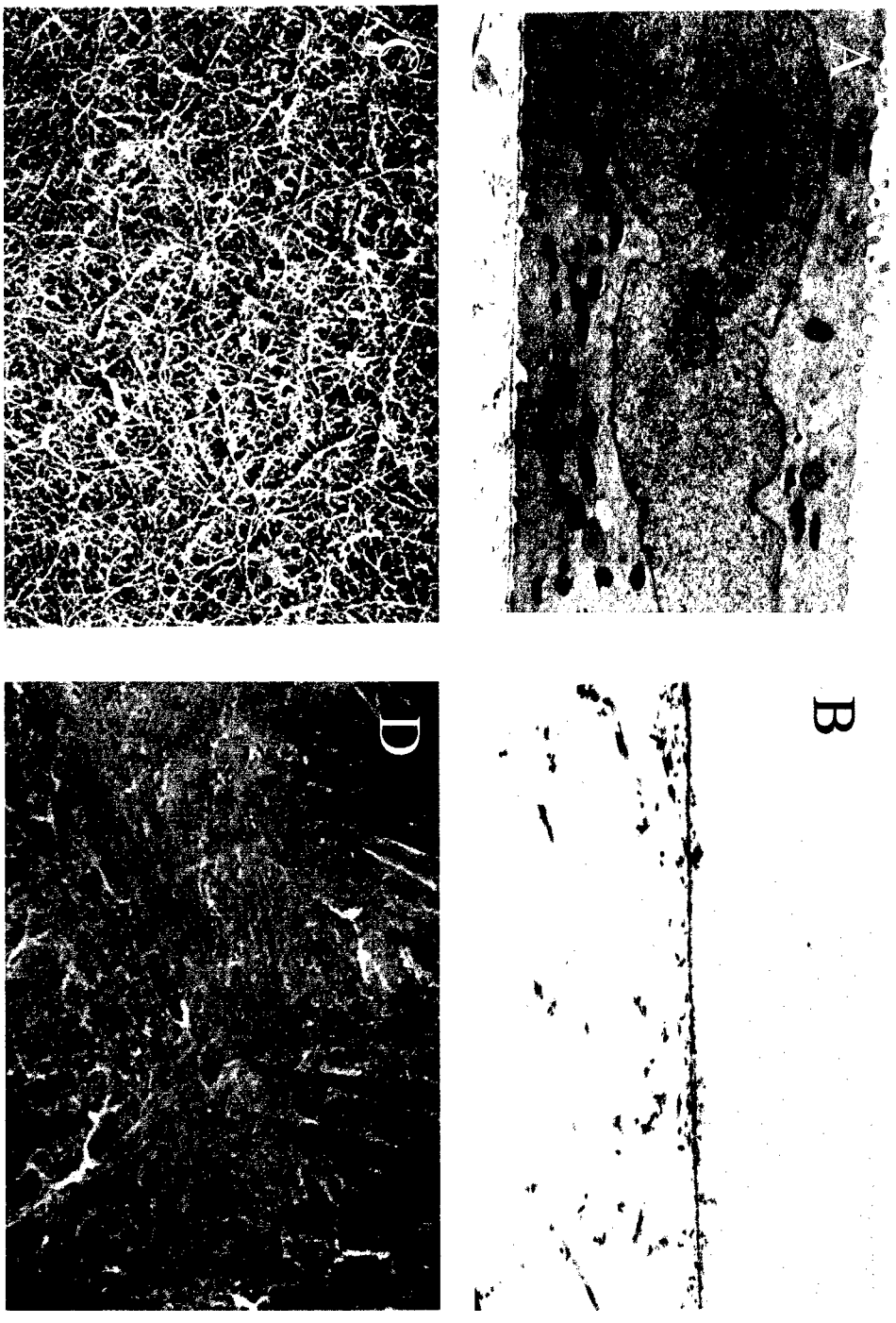
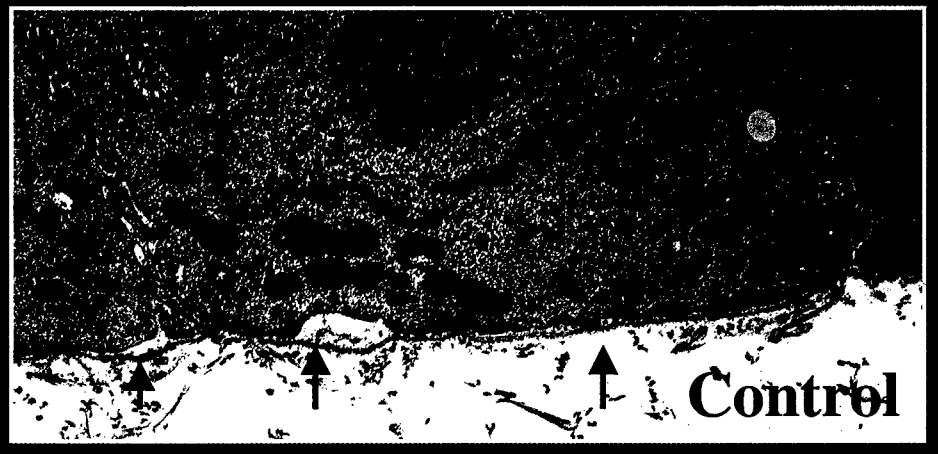


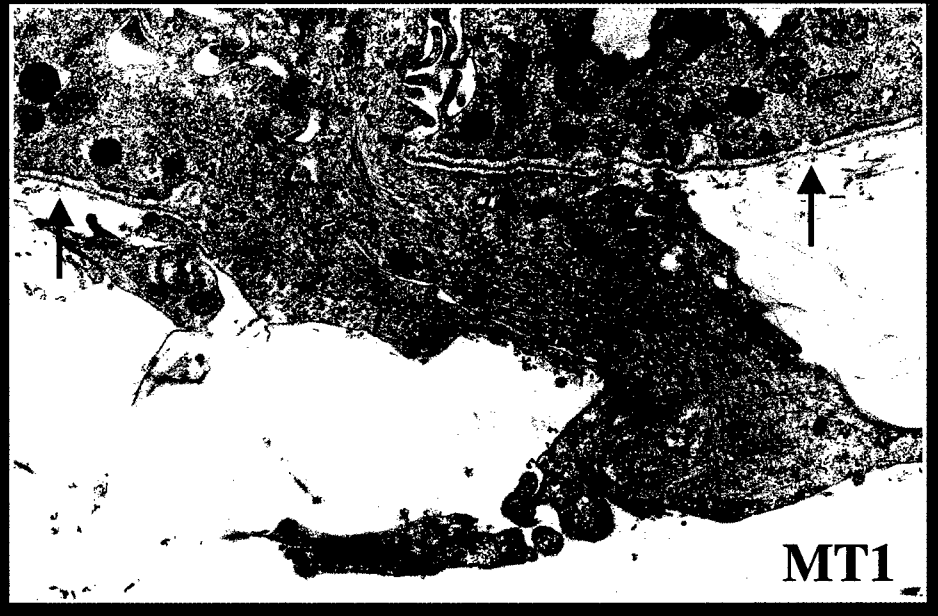
Figure 3

Stromelysin-1 Does Not Confer Basement Membrane-Invasive Activity

Figure 4

Basement Membrane Invasion Mediated By MT1-MMP





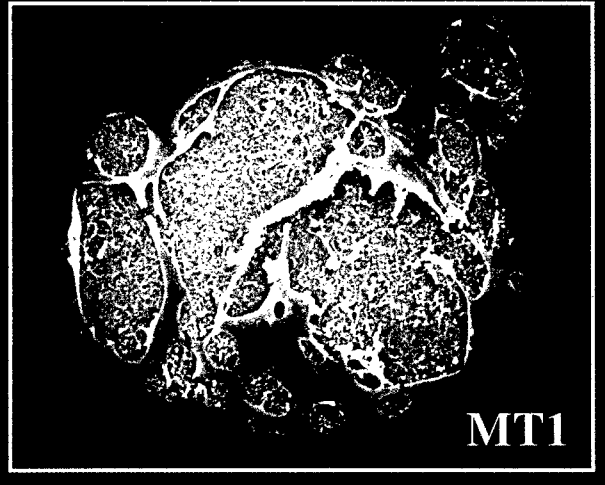
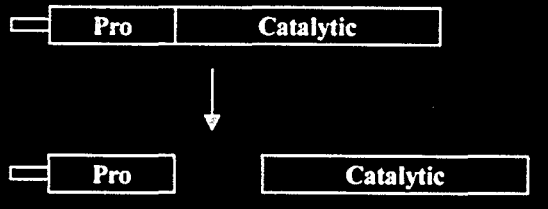
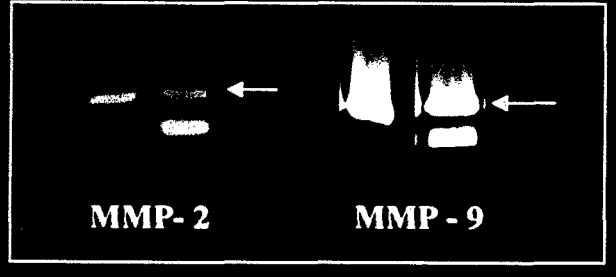


Figure 5

Fully Active MMP-2 and MMP-9 Do Not Invade Basement Membrane

Proprotein convertase recognition sequence (RXXR) inserted between Pro domain and catalytic domain - generates active enzyme.



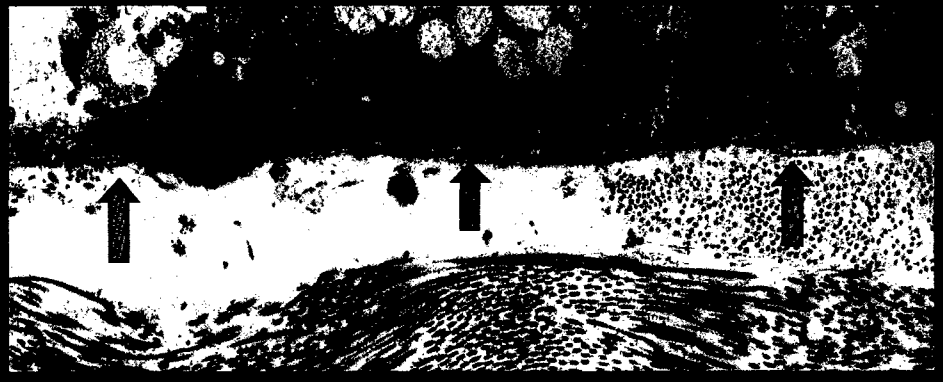


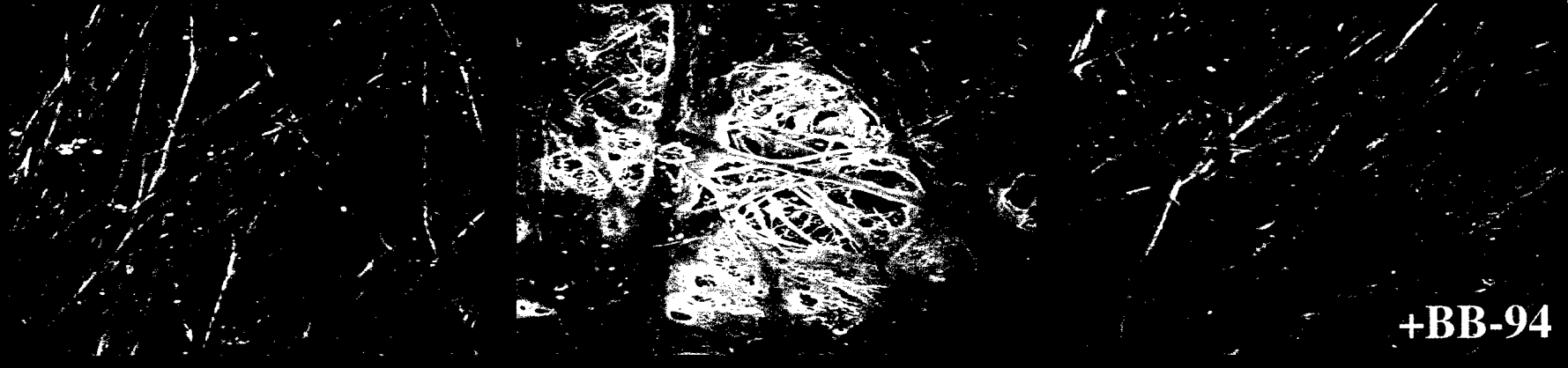
MMP - 2

MMP - 9

Figure 6

Basement Membrane Invasion By MT1-MMP





Control

MT1-MMP

Figure 7

MT1-MMP Regulates Basement Membrane Invasion of MCF-7 Cells

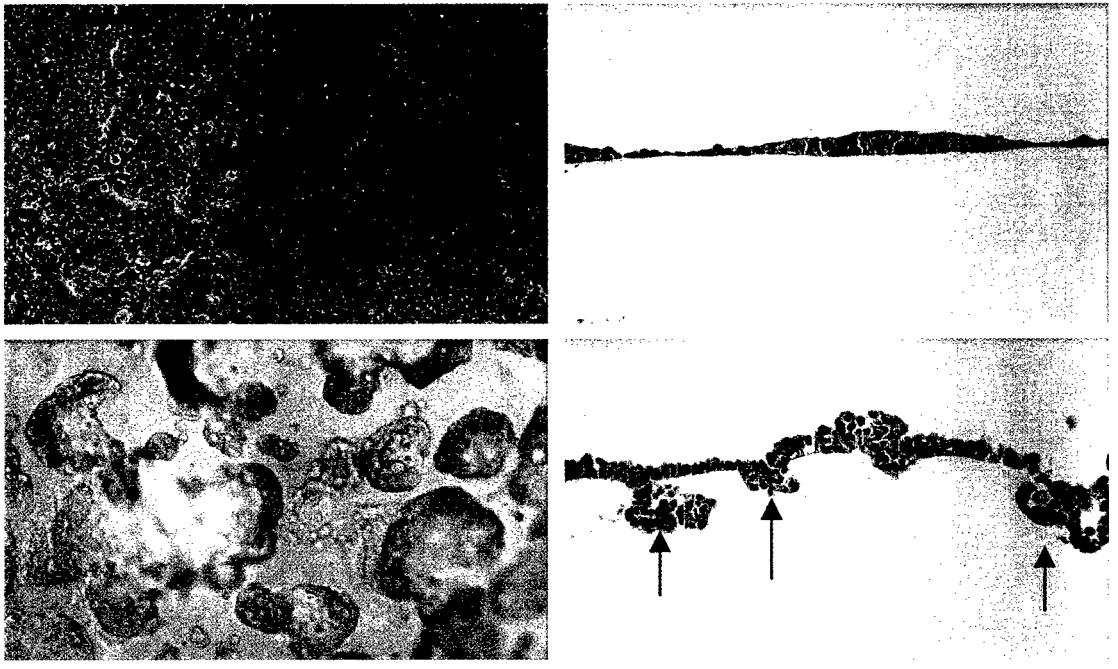
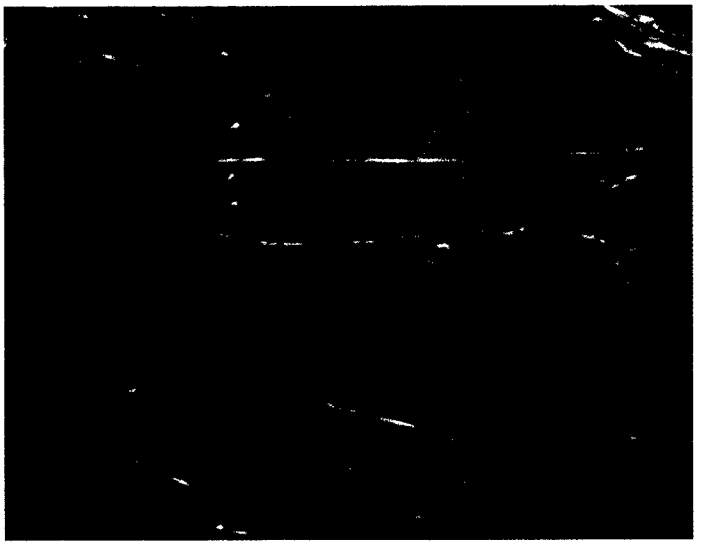
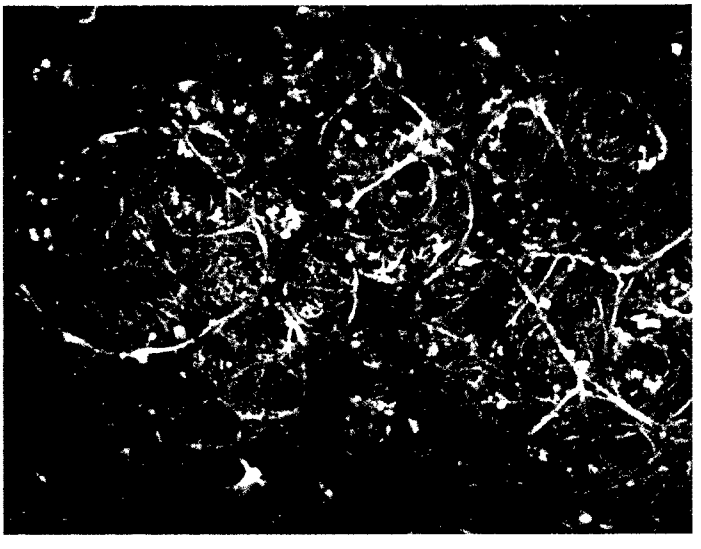


Figure 8

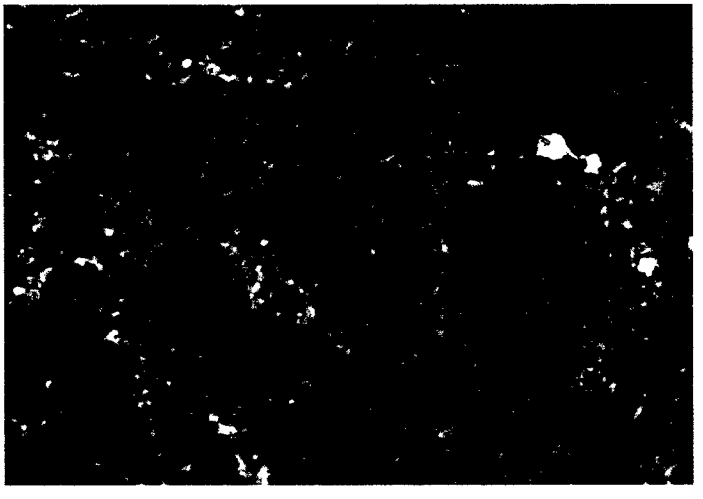
MT1-MMP-Positive MB-231 Cells Display Invasive Activity



Control



MB-231



MB-231
+ TIMP-2

Figure 9

APPENDIX

CHIP	PROBESET	Inv A	Inv C	Inv D	Lac J	Lac K	Lac N	DESC
A	108235_s	3894	4535	3521	915	643	652	Mus musculus clusterin mRNA, complete cds.
A	112447_s	1861	2287	2039	943	297	297	Mus musculus insulin-like growth factor binding protein 5 (IGFBP5) mRNA, complete cds.
A	AA709861_f	1694	1926	1090	705	390	442	vt35a01.r1 Barstead Mu proximal colon MPLRB6 Mus musculus cDNA clone 1165032 5', mRNA sequence.
B	Msa.1271.0_s	1168	1258	1105	479	260	280	House Mu; Musculus domesticus mRNA for lactoferrin, complete cds
A	d11468_s	1047	1148	1114	181	358	417	Mu gene for immunoglobulin alpha heavy chain, switch region and constant region complete sequence.
A	u73004_s	799	778	695	186	89	105	Mus musculus secretory leukocyte protease inhibitor mRNA, complete cds.
B	x52886_s	768	628	682	337	161	145	M.musculus mRNA for cathepsin D.
B	ET62985_f	730	995	787	169	293	364	M.musculus mRNA (1B5) for IgA V-D-J-heavy chain.
B	X00496_s	725	600	593	339	69	33	Mu Ia-associated invariant chain (Ii) mRNA fragment.
B	Msa.22134.0_s	534	480	465	312	69	66	Homologous to sp P13983: EXTENSIN PRECURSOR (CELL WALL HYD
B	Msa.739.0_s	526	643	632	307	127	142	C57BL/6J ob/ob haptoglobin mRNA, complete cds
B	X62940_s	499	421	439	196	98	67	M.musculus TSC-22 mRNA.
A	U62386_s	483	393	352	39	38	54	Mus musculus immunoglobulin heavy and light chain variable region mRNA, complete cds.
A	M73329_s	481	520	477	342	44	98	Mu phospholipase C-alpha (PLC-alpha) mRNA, complete cds.
B	w08453_s	417	293	436	191	55	67	W08453 mb50a09.r1 Soares Mu p3NMF19.5 Mus musculus cDNA clone 332824 5' similar to gb:S65738 DESTRIN (HUMAN); TIGR cluster TC34375
B	x67141	415	778	805	383	59	35	M.musculus Pva mRNA for parvalbumin.
B	X13605_s	406	274	364	219	50	40	Murine mRNA for replacement variant histone H3.3.
B	ET63295_f	385	318	326	31	126	126	M.domesticus IgM variable region.)PIR:S26747 (Ig heavy chain J region JH4 - Mu
A	aa711217_s	349	372	391	230	46	72	vt70b10.r2 Barstead Mu irradiated colon MPLRB7 Mus musculus cDNA clone 1176475 5' similar to SW:NB2M_BOVIN Q02365 NADH-UBIQUINONE OXIDOREDUCTA
B	x51438_s	342	185	290	167	22	32	Mu mRNA for vimentin.
B	Msa.6056.0_s	324	353	413	215	19	28	Homologous to sp P09912: INTERFERON-INDUCED PROTEIN 6-16 PRECURSOR (IFI-6-16).
B	Msa.41264.0_s	309	494	535	239	-8	-11	Homologous to sp P04462: MYOSIN HEAVY CHAIN, PERINATAL SKELETAL MUSCLE (FRAGMENT).
B	ET61925_f	299	364	344	72	92	118	Mus musculus anti-DNA immunoglobulin light chain IgG, antibody 363s.71, partial cds.
B	ET62705_f	291	198	200	39	79	77	Mus musculus anti-DNA antibody heavy chain variable region mRNA, partial cds.
B	Msa.22488.0_s	244	194	221	106	32	30	Homologous to sp Q02765: CATHEPSIN S PRECURSOR (EC 3.4.22.27).
B	x06115_s	226	128	168	95	45	33	Mu mRNA for E-cadherin (= uvomorulin, = L-CAM, = cell-CAM 120/80, = Arc-1).
B	x61433_s	223	211	227	120	49	22	M.musculus mRNA for sodium/potassium ATPase beta subunit.
B	AFFX-MURINE_b1	216	128	169	87	10	20	MURINE_b1
A	J04992_f	200	324	312	212	23	30	Mu fast fiber troponin I mRNA, complete cds.
B	ET63288_f	194	172	170	26	67	65	M.domesticus IgM variable region.)PIR:PH0975 (Ig heavy chain V region (clone 163.72) - Mu (fragment)
B	x14194_s	190	131	193	51	1	-7	Mu mRNA for entactin.
B	ET63358_f	178	174	161	17	29	27	M.domesticus IgK variable region.)PIR:PH1046 (Ig light chain V region (clone 202.9) - Mu (fragment))PIR:PH1048 (Ig light chain V region (clone 165.49) - Mu (fragment))PI
B	Msa.88.0_s	173	150	153	109	27	21	Mu mRNA for osteoblast specific factor 2 (OSF-2)
B	Msa.41890.0_f	171	297	276	170	22	25	Homologous to sp P10469: TROPOMYOSIN ALPHA CHAIN, SMOOTH MUSCLE (FRAGMENT).
B	Msa.2614.0_g	169	187	178	87	40	45	Mu mRNA for properdin (AA 5 - 441)
B	w54482	154	60	103	44	19	25	W54482 md08e09.r1 Soares Mu embryo NbME13.5 14.5 Mus musculus cDNA clone 367816 5' similar to WP:T12A2.7 CE01400 ;. TIGR cluster TC15447
B	ET62941_f	152	254	258	66	68	83	M.musculus antibody light chain variable region (318bp).
B	Msa.2642.0_f	144	284	216	185	4	1	Mus musculus adenine nucleotide translocase-1 (Ant1) mRNA, complete cds
A	m27009_s	143	101	88	57	-17	-15	Mu alpha-1 acid glycoprotein (Agp-2) mRNA, complete cds.

B	x62600_s	139	54	79	36	6	-10	M.musculus mRNA for C/EBP beta.
B	X64837_s	139	88	83	66	26	6	M.musculus Oat mRNA for ornithine aminotransferase.
A	aa690738_s	138	118	114	29	-32	-31	vu57b03.r1 Soares Mu mammary gland NbMMG Mus musculus cDNA clone 1195469 5', mRNA sequence.
A	aa260736_s	130	98	93	67	15	14	AA260736 va02a11.r1 Soares Mu lymph node NbMLN Mus musculus cDNA clone 721724 5' TIGR cluster TC22696
B	X15591_s	128	98	110	54	14	7	Mu ctla-2-alpha mRNA, homolog. to cysteine protease proregion.
B	ET61285_f	126	70	74	6	25	24	Mus musculus anti-DNA immunoglobulin heavy chain variable region, clone 4B2, partial cds.
A	aa265871	124	116	117	103	-7	6	AA265871 mz70h03.r1 Soares Mu lymph node NbMLN Mus musculus cDNA clone 718805 5' TIGR cluster TC34843
B	Msa.21652.0_f	123	82	114	51	10	8	Homologous to sp P09568: LYMPHOCYTE ANTIGEN LY-6C.2/LY-6C.1 PRECURSOR.
B	w30230_g	123	40	78	40	8	7	W30230 mc26f11.r1 Soares Mu p3NMF19.5 Mus musculus cDNA clone 349677 5' TIGR cluster TC26484
B	w82831_i	122	54	83	16	-8	-5	W82831 mf07e10.r1 Soares Mu p3NMF19.5 Mus musculus cDNA clone 404394 5' TIGR cluster TC23212
A	aa254768	112	94	99	48	0	-5	AA254768 mz75h08.r1 Soares Mu lymph node NbMLN Mus musculus cDNA clone 719295 5'
B	Msa.728.0	111	117	159	82	-16	-15	M.musculus glucose transporter 2 mRNA, complete cds
A	M60474_f	110	105	94	51	2	7	Mu myristoylated alanine-rich C-kinase substrate (MARCKS) mRNA, complete cds.
B	x66402_s	109	69	77	25	-3	-10	M.musculus mRNA for stromelysin 1.
A	aa684097	107	110	103	68	10	16	vm66d02.s1 Knowles Solter Mu 2 cell Mus musculus cDNA clone 1003203 5', mRNA sequence.
A	u34920_s	107	82	85	18	-21	-18	Mus musculus white homolog (white) mRNA, complete cds.
B	Msa.39985.0_f	107	229	259	141	-8	-7	Homologous to sp P49438: TROPOMYOSIN ALPHA CHAIN, MAJOR BRAIN ISOFORM.
A	c75983_rc_f	99	112	165	46	29	29	C75983 Mu 3.5-dpc blastocyst cDNA Mus musculus cDNA clone J0001E09 3' similar to Unannotatable data, mRNA sequence.
B	ET61749_f	97	80	73	18	27	23	Mus musculus Ig 6C3.B8 heavy chain mRNA, specific for rat (Mu) cytochrome c, partial cds.
A	M27008	95	63	72	76	3	-18	Mu alpha-1 acid glycoprotein (Agp-1B) mRNA, complete cds.
A	aa690872_f	93	73	80	68	-5	0	vt32c11.r1 Barstead Mu proximal colon MPLRB6 Mus musculus cDNA clone 1164788 5' similar to gb:D00762 PROTEASOME COMPONENT C8 (HUMAN);, mRNA sequ
B	Msa.43191.0_s	92	74	75	54	-4	3	M.musculus integrin associated protein mRNA, complete CDS (EXTRACTED 3'UTR)
B	Msa.10564.0	90	58	68	24	16	21	Homologous to sp P09117: FRUCTOSE-BISPHOSPHATE ALDOLASE (EC 4.1.2.13) C (BRAIN).
A	D49744_s	87	85	89	59	3	4	Mu mRNA for farnesyltransferase alpha subunit, complete cds.
A	M74227_s	85	71	80	37	9	7	Mu cyclophilin C (cyp C) mRNA, complete cds.
B	Msa.727.0_s	82	55	51	33	2	11	Mu glutathione S-transferase (GST Yc) mRNA, complete cds
A	M63695_s	82	48	54	7	-25	-23	Mu CD1.1 mRNA, complete cds.
B	x56548_s	80	52	69	22	-12	-10	M.musculus Np-b mRNA for purine-nucleoside phosphorylase.
A	D50032_s	79	73	47	18	13	9	Mu mRNA for TGN38B, complete cds.
A	U25844_s	79	77	63	27	-19	-32	Mus musculus serine proteinase inhibitor (SPI3) mRNA, complete cds.
A	U58883_s	79	59	38	44	-7	-8	Mus musculus putative SH3-containing protein SH3P12 mRNA, patrtial cds.
B	ET61918_f	79	95	113	6	27	42	Mus musculus anti-DNA immunoglobulin light chain IgM mRNA, antibody 363p.202, partial cds.
A	AA274696_s	78	51	63	38	6	-3	AA274696 vc05b03.r1 Soares Mu lymph node NbMLN Mus musculus cDNA clone 765581 5' TIGR cluster TC38052
B	x83932_s	73	84	134	69	7	8	M.musculus mRNA for ryanodine receptor type 1.
B	z11997_s	70	60	87	-1	-21	-22	M.musculus mRNA for non-histone chromosomal high-mobility group 1 protein.
A	r75131_rc_s	69	117	102	66	-1	-7	R75131 MDB1078 Mu brain; Stratagene Mus musculus cDNA 3'end. TIGR cluster TC17356
B	ET62942_f	69	82	79	9	24	28	M.musculus antibody light chain variable region (324bp).
A	AA170444	69	65	56	34	15	12	AA170444 ms90f10.r1 Soares Mu 3NbMS Mus musculus cDNA clone 618859 5' similar to SW:UBAL_HUMAN P41226 UBIQUITIN-ACTIVATING ENZYME E1 HOMOL
A	aa253918	69	73	91	13	-25	-25	AA253918 mw07h06.r1 Soares Mu 3NME12 5 Mus musculus cDNA clone 670043 5' similar to gb:Z21507 ELONGATION FACTOR 1-DELTA (HUMAN); TIGR cluster TC
A	AA259399_s	67	54	54	63	-5	-1	AA259399 va51b02.r1 Soares Mu 3NME12 5 Mus musculus cDNA clone 734859 5' TIGR cluster TC35817

B	Msa.41380.0_s	66	166	181	95	6	12	Homologous to sp Q08043: ALPHA-ACTININ 3, SKELETAL MUSCLE ISOFORM (F-ACTIN CROSS LINKING PROTEIN).
B	v00727_s	61	26	69	21	14	10	Mu c-fos oncogene.
A	j05479_s	60	50	50	28	-2	4	Mu calcineurin catalytic subunit mRNA, complete cds.
A	AA597258	60	46	50	44	2	3	vo36a09.r1 Barstead Mu irradiated colon MPLRB7 Mus musculus cDNA clone 1051960 5'. mRNA sequence.
A	L04961_s	58	44	49	27	-15	3	Mu nuclear-localized inactive X-specific transcript (Xist) mRNA.
B	Msa.3557.0_s	56	70	59	29	-8	-5	Homologous to sp P36955: PIGMENT EPITHELIUM-DERIVED FACTOR PRECURSOR (PEDF) (EPC-1).
A	AA203803_s	56	56	44	39	9	2	AA203803 mu61b05.r1 Soares Mu lymph node NbMLN Mus musculus cDNA clone 643857 5' TIGR cluster TC25395
A	aa500554_s	49	55	59	32	13	3	AA500554 vi86a01.r1 Stratagene Mu skin (#937313) Mus musculus cDNA clone 919080 5' TIGR cluster TC39733
A	af023463_s	49	54	58	38	8	6	Mus musculus peroxisomal phytanoyl-CoA alpha-hydroxylase (PAHX) mRNA, complete cds.
B	ET62260_f	43	160	162	22	30	39	Mus musculus immunoglobulin light chain variable region mRNA, partial cds.
A	M74495_s	41	87	88	49	5	4	Mu adenylosuccinate synthetase mRNA, complete cds.
A	c76162_rc_f	32	75	104	12	11	9	C76162 Mu 3.5-dpc blastocyst cDNA Mus musculus cDNA clone J0004G06 3' similar to Rat insulin-I (ins-1) gene, mRNA sequence.